# 1
# Amino-acids

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The past twelve months have witnessed interesting developments in amino-acid chemistry, but pride of place must be taken by the isolation, characterization, and synthesis of the amino-acid derivative from phenylalanine t-RNA. This work also reflects the growing sophistication in the application of physical methods in general. The interesting advances reported last year on asymmetric synthesis have been extended, and a staggering number of new amino-acids continues to be synthesized for a variety of reasons. The established pattern of coverage for this chapter is maintained, attention being focused on a broad selection of topics with the significant developments highlighted where necessary.

## 1 Naturally Occurring Amino-acids

**A. Occurrence of Known Amino-acids.**—A large number of papers which are concerned with the free amino-acid content of a wide variety of living organisms is published annually. Since the emphasis for the majority of this work is on biological aspects, it is not considered appropriate to cover them in this section and only those amino-acids which are rarely encountered or are interesting from the chemical viewpoint have been included.

The presence of amino-acids in extra-terrestrial material as well as their synthesis under simulated prebiotic conditions (see Section 2B) has attracted a considerable amount of attention. Examination of samples from the Apollo 11 and Apollo 12 missions has revealed extremely low concentrations of amino-acids (*ca.* 20—70 p.p.b.);[1-3] ultra-sensitive analytical techniques were employed for these investigations and, because of the presence of a considerable number of non-protein amino-acids, the investigators maintain that they are not due to terrestial contamination. Similar conclusions have been made concerning the presence of amino-acids in the Murray [4] and

[1] B. Nagy, J. E. Modzeleski, V. E. Modzeleski, M. A. J. Mohammad, L. A. Nagy, W. M. Scott, C. M. Drew, J. E. Thomas, R. Ward, P. B. Hamilton, and H. C. Urey, *Nature*, 1971, **232**, 94.
[2] K. Harada, P. E. Hara, C. R. Windsor, and S. W. Fox, *Science*, 1971, **173**, 433.
[3] C. W. Gehrke, R. W. Zumwalt, D. L. Stalling, D. Roach, W. A. Aue, C. Ponnamperuma, and K. A. Kvenvolden, *J. Chromatog.*, 1971, **59**, 305.
[4] J. G. Lawless, K. A. Kvenvolden, E. Peterson, C. Ponnamperuma, and C. Moore, *Science*, 1971, **173**, 626.

Murchinson [5] meteorites. In the case of the Murray meteorite, seventeen amino-acids were identified of which seven were conclusively shown to be racemic and eleven to be non-protein in origin. An earlier report [6] that the amino-acid content of the Orgueil meteorite was due solely to terrestrial contamination has been questioned, and it is now suggested that there are amino-acids indigenous to the meteorite in addition to those present as contaminants.[7]

The presence of amino-acids in the North Atlantic ocean has been the subject of a detailed examination and the distribution appears to be non-uniform and varies qualitatively with depth.[8] In marine sediments the degree of racemization of free amino-acids shows a progressive increase with the age of the sediment,[9, 10] and the ratio of *allo*-isoleucine to leucine is a reliable indicator of age for samples less than 400 000 years old. In samples older than about $15 \times 10^6$ years the amino-acids are completely racemic.

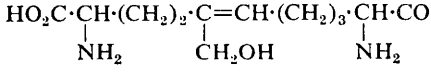
The stereochemistry of the α-hydroxy-analogue of cysteine present in the urine of certain mentally retarded patients has been established by comparison with synthetic material.[11] The work reported initially last year on the isolation of the methylated derivatives of arginine and lysine has been extended and it has been noted that, in patients with malignant tumours, the relative urinary level of *guanidino-NN*-dimethylarginine to that of arginine is markedly increased.[12] Bovine and rat brain tissue has been shown to contain appreciable amounts of $N^G$-monomethylarginine as well as $N^G N'^G$-dimethylarginine.[13]

Further spectral evidence on the aldol condensation product isolated on alkaline hydrolysis of elastin provides strong support [14] for the previously assigned structure (1).

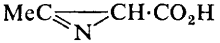
Interest in plants containing L-Dopa continues, and a widespread investigation has shown that several *Mucuna* species contain up to 5% of this important amino-acid.[15] The neurotoxin α-amino-β-oxalylaminopropionic acid has been isolated from *Lathyrus sativus* [16] and the major alkaloid of *Aotus subglauca* has been identified as *S*-(+)-$N^\alpha$-methyltryptophan methyl ester.[17] The previously unidentified amino-acid from *Peganum harmala* is

[5] J. R. Cronin and C. B. Moore, *Science*, 1971, **172**, 1327.
[6] J. Oro, S. Nakaparksin, H. Lichenstein, and E. Gil-Av, *Nature*, 1971, **230**, 107.
[7] J. G. Lawless, K. A. Kvenvolden, E. Peterson, and C. Ponnamperuma, *Nature*, 1972, **234**, 66.
[8] R. Pocklington, *Nature*, 1971, **230**, 374.
[9] R. O. Brinkhurst, K. E. Chua, and E. Batoosingh, *Limnol. Oceanogr.*, 1971, **16**, 555.
[10] J. Wehmiller and P. E. Hare, *Science*, 1971, **173**, 907.
[11] M. Wälti and D. B. Hope, *J. Chem. Soc.* (*C*), 1971, 2326.
[12] S. Akazawa, *Osaka Daigaku Igaku Zasshi*, 1970, **22**, 461.
[13] T. Nakajima, Y. Matsuoka, and Y. Kakimoto, *Biochim. Biophys. Acta*, 1971, **230**, 212.
[14] G. Crombie, B. Faris, P. M. Gallop, and C. Franzblau, *Biochemistry*, 1971, **10**, 4145.
[15] E. A. Bell, J. R. Nulu, and C. Cone, *Phytochemistry*, 1971, **10**, 2191.
[16] K. Bahadar and S. P. Billa, *Indian J. Appl. Chem.*, 1970, **33**, 168.
[17] S. R. Johns, J. A. Lamberton, and A. A. Sioumis, *Austral. J. Chem.*, 1971, **24**, 439.

established as L-4-hydroxypipecolic acid.[18] D-Pipecolic acid, as well as L-*threo*- and D-*erythro*-$\alpha\beta$-diaminobutyric acid, has been isolated on acid hydrolysis of the antibiotic amphomycin,[19] and griselimycin afforded, *inter alia*, 4-*trans*-4-methyl-L-proline and *N*-methyl-D-leucine on hydrolysis.[20]

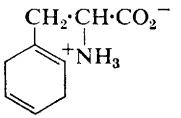


(1)



(2)

**B. New Natural Free Amino-acids.**—The novel azirine amino-acid (2) isolated from a strain of *Streptomyces aureus*, and appropriately named azirinomycin, exhibits broad-spectrum activity *in vitro* against both Gram positive and negative bacteria.[21] It is unstable especially in concentrated form and was identified by spectral analysis and by conversion on catalytic hydrogenation into L-$\alpha$-aminobutyric acid.[22] L-2,5-Dihydrophenylalanine (3), which had previously been synthesized by a Birch reduction of L-phenylalanine,[23] is an antimetabolite of L-phenylalanine produced by an unidentified *streptomyces*.[24] A related compound, anticapsin (4), is produced by a strain of *Streptomyces griseoplanus* [25] and is presumably derived from L-tyrosine by a similar reduction with subsequent epoxidation.



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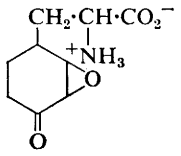
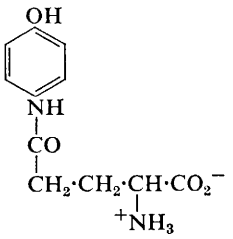


(4)

The increasing application of mass spectrometry as a valuable tool for characterization is apparent and has been employed for many of the amino-acids described in this section. Trimethylsilylation is still the most commonly employed procedure for enhancing the volatility of amino-acids containing a number of polar substituents and has been successfully applied in characterizing L-*threo*-$\alpha$-amino-$\beta\gamma$-dihydroxybutyric acid.[26] The new compound (5), which has been isolated from the common mushroom, affords on oxidation an unstable quinone, also present in the organism.[27]

[18] V. U. Ahmad and M. A. Khan, *Phytochemistry*, 1971, **10**, 3339.
[19] M. Bodanszky, N. C. Chaturvedi, J. A. Scozzie, R. K. Griffith, and A. Bodanszky, *Antimicrobial Agents and Chemotherapy*, 1969, 135.
[20] B. Terlain and J. P. Thomas, *Bull. Soc. chim. France*, 1971, 2349.
[21] E. O. Stapley, D. Hendlin, M. Jackson, and A. K. Miller, *J. Antibiotics*, 1971, **24**, 42.
[22] T. W. Miller, E. W. Tristram, and F. J. Wolf, *J. Antibiotics*, 1971, **24**, 48.
[23] M. L. Snow, C. Lawinger, and C. Ressler, *J. Org. Chem.*, 1968, **33**, 1774.
[24] J. P. Scannell, D. L. Pruess, T. C. Demny, T. Williams, and A. Stempel, *J. Antibiotics*, 1970, **23**, 618.
[25] R. Shah, N. Neuss, M. Gorman, and L. D. Boeck, *J. Antibiotics*, 1970, **23**, 613.
[26] J. W. Westley, D. L. Pruess, L. A. Volpe, T. C. Demny, and A. Stempel, *J. Antibiotics*, 1971, **24**, 330.
[27] R. F. Weaver, K. V. Rajagopalan, P. Handler, D. Rosenthal, and P. W. Jeffs, *J. Biol. Chem.*, 1971, **246**, 2010.



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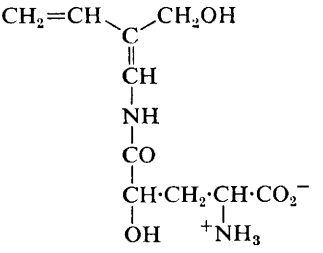
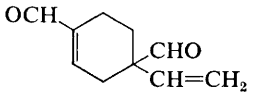


(6)

Glutamic acid derivatives have also been isolated from a number of plant sources. Pinnatanine (6), a new amino-acid isolated from the European bladder nut [28] (*Staphylea pinnata*), affords on acid hydrolysis L-*allo*-γ-hydroxyglutamic acid, ammonia, and compound (7), the structure of which has been confirmed by synthesis. It is suggested that (7) arises *via* a Diels–Alder dimerization of the transient 2-methylenebut-3-enal initially formed in the hydrolysis. In the course of an investigation on nitrogen metabolism in tobacco plants a new amino-acid, nicotianamine, was isolated and the structure (8) was assigned on the basis of extensive chemical degradation and spectral analysis.[29] Azetidine-2-carboxylic acid had previously been obtained from a considerable number of plant species.[30]



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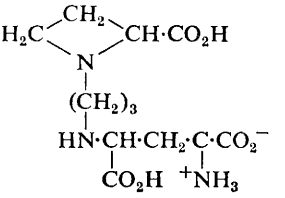

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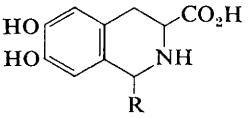
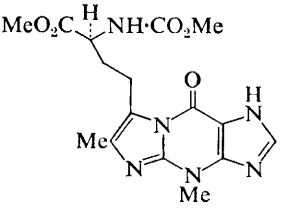


(9) R = H
(10) R = Me



(11)

The isoquinoline derivative (9) has been isolated from the plant *Mucuna mutisiana* [15] which also contains appreciable amounts of L-Dopa, and it

[28] M. D. Grove, M. E. Daxenbichler, D. Weisleder, and C. H. VanEtten, *Tetrahedron Letters*, 1971, 4477.
[29] M. Noma, M. Noguchi, and E. Tamaki, *Tetrahedron Letters*, 1971, 2017.
[30] L. Fowden, *Adv. Enzymol.*, 1967, **29**, 89.

appears likely that (9) is derived from L-Dopa by condensation with formaldehyde. The corresponding derivative (10), formed from L-Dopa and acetaldehyde, had previously been isolated.[31]

The urine of homocystinuric patients has been shown to contain *S*-(3-hydroxy-3-carboxy-n-propylthio)- and *S*-(2-hydroxy-2-carboxyethylthio)-homocystine[32] in addition to the α-hydroxy-analogue of cystine. Further work on the phaemelanin pigments has been reported, and an isomer of trichosiderin C has been isolated and characterized.[33]

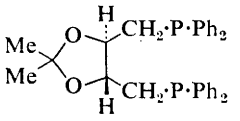
**C. New Amino-acids from Hydrolysates.**—The most notable achievement in this field is undoubtedly the characterization and subsequent synthesis (see Section 2C) of the fluorescent Y base present in phenylalanine t-RNA derived from yeast, wheat germ, and rat liver. It represents a significant triumph for the application of physical methods; the structure (11) was established on 300 μg of material mainly on the interpretation of the results obtained from high-resolution mass spectrometry and n.m.r. spectroscopy.[34]

Full details on the structures of the novel piperazic acid derivatives from the antibiotic monamycin[35] and the guanidine amino-acid viomycidine from viomycin[36] have been reported. β-Hydroxyhistidine has been isolated on the acid hydrolysis of the antibiotic bleomycin.[37]

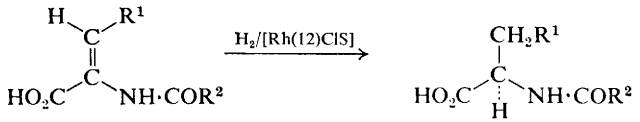
## 2 Chemical Synthesis and Resolution of Amino-acids

**A. Introduction and General Methods.**—The interest in asymmetric syntheses of amino-acids continues unabated and a valuable article covering the literature up to 1969 has been published.[38] In addition those syntheses which involve enantioselective catalytic hydrogenation have also been reviewed.[39] In this respect it is of considerable interest to note that this year has seen the first significant application of homogeneous asymmetric catalysis to amino-acid synthesis.[40] The rhodium complex of the chiral diphosphine (12) readily reduces *N*-acyldehydroamino-acids (13) to the corresponding (*R*)-*N*-acylamino-acids in high yield and with an optical efficiency of about 70%. The relatively high stereoselectivity is ascribed to the conformational rigidity of the diphosphine chelating the rhodium, together with the participation of the acid function of the substrate.

[31] P. Müller and H. R. Schütte, *Z. Naturforsch.*, 1968, **236**, 491.
[32] H. Kodama, S. Ohmori, M. Suzuki, S. Mizuhara, T. Oura, G. Isshiki, and I. Uemura, *Physiol. Chem. Phys.*, 1971, **3**, 81.
[33] G. Prota, A. Suarato, and R. A. Nicolaus, *Experientia*, 1971, **27**, 1145.
[34] K. Nakanishi, N. Furutachi, M. Funamizu, D. Grunberger, and I. B. Weinstein, *J. Amer. Chem. Soc.*, 1970, **92**, 7617.
[35] C. H. Hassall, Y. Ogihara, and W. A. Thomas, *J. Chem. Soc.* (*C*), 1971, 522.
[36] G. Büchi and J. A. Raleigh, *J. Org. Chem.*, 1971, **36**, 871.
[37] T. Takita, T. Yoshioka, Y. Muraoka, K. Maeda, and H. Umezawa, *J. Antibiotics*, 1971, **24**, 795.
[38] J. D. Morrison and H. S. Mosher, 'Asymmetric Organic Reactions', Prentice-Hall, New Jersey, 1971.
[39] Y. Izumi, *Angew. Chem., Internat. Edn.*, 1971, **12**, 871.
[40] T. P. Dang and H. B. Kagan, *Chem. Comm.*, 1971, 481.

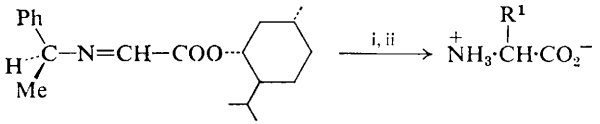


(12)



(13)

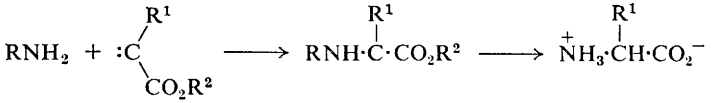
The inherent problem of conformational mobility of the substrate in all asymmetric syntheses which was referred to in last year's Report is probably the cause of the relatively low optical efficiency of the new synthesis [41] outlined in Scheme 1 and of the asymmetric reduction of dehydroamino-acid



Reagents: i, $R^1MgX$; ii, $H^+$

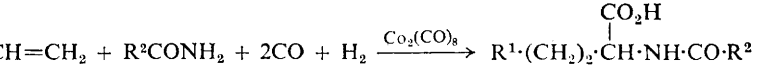
**Scheme 1**

peptides.[42] Unfortunately, the ingenious synthesis of amino-acids by the insertion of a carbene into an N—H bond of an optically active amine [43] (Scheme 2) also gives low optical efficiency, presumably for the same reason.



**Scheme 2**

A general synthesis has been reported [44] which employs a carbonylation reaction and is essentially a variation of the hydroformylation reaction and the Oxo process (Scheme 3). Although the products are completely racemic



**Scheme 3**

[41] J. Fiaud and H. B. Kagan, *Tetrahedron Letters*, 1971, 1019.
[42] M. Nakayama, G. Maeda, T. Kaneko, and H. Katsura, *Bull. Chem. Soc. Japan*, 1971, **44**, 1150.
[43] J. F. Nicoud and H. B. Kagan, *Tetrahedron Letters*, 1971, 2065.
[44] H. Wakamatsu, J. Uda, and N. Yamakami, *Chem. Comm.*, 1971, 1540.

the yields are reasonable and it is claimed that the reaction has a wide application to amino-acid synthesis. The starting materials are readily available and the synthesis reflects the growing commercial interest in the large-scale chemical production of amino-acids.

**B. Synthesis under Simulated Prebiotic Conditions.**—It is now well established that amino-acids are formed when a simulated primitive atmosphere is subjected to high temperature, u.v. irradiation, or high-frequency discharge, or any combination of these conditions. Recent work has centred on more rigorous analysis of the reaction mixtures and identification of amino-acids with functional groups in the side-chain.[45, 46] Irradiation of mixtures of methane, ammonia, hydrogen sulphide, and water produces mixtures which contain either cysteine or cystine depending on the reaction conditions[47] and similar experiments using high-frequency discharge produce mixtures in which histidine has been conclusively identified.[48, 49] The preferential adsorption of the L-isomer of racemic amino-acids on Kaolinite templates has been demonstrated [50] and is of particular interest in connection with the natural predominance of L-amino-acids.

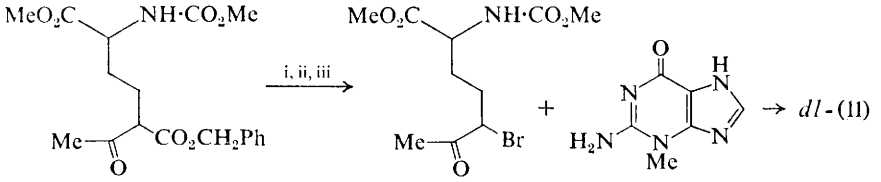
**C. Protein and Other Naturally Occurring Amino-acids.**—Many of the syntheses described in this section have been achieved by standard procedures, and therefore only the salient features of the more important will be presented.

The various methods for the synthesis of glycine have been reviewed [51] and a large-scale preparation of ornithine from glutamic acid has been reported.[52] A new synthesis of threonine from the glycine copper complex and acetaldehyde has been described [53] together with a detailed investigation of the course of this reaction. Interest in the synthesis of L-Dopa and related compounds (Section 2F) continues, and two further syntheses [54, 55] are now available.

The full details of the syntheses of capreomycidine,[56] indospicine,[57] and the piperazic acid derivatives [58] from the antibiotic monamycin, initially reported in preliminary form, have now been published.

[45] D. Yoshino, R. Hayatsu, and E. Anders, *Geochim. Cosmochim. Acta*, 1971, **35**, 927, 939.
[46] V. Marshall and A. Bennett, *Proc. Indian Acad. Sci.*, 1970, **80**, 369.
[47] B. N. Khare and C. Sagan, *Nature*, 1971, **232**, 577.
[48] S. Yuasa, M. Ishigami, Y. Honda, and K. Imahori, *Sci. Rep. Osaka Univ.*, 1970, **19**, 33.
[49] S. Yuasa, M. Yamamoto, Y. Honda, M. Ishigami, and K. Imahori, *Sci. Rep. Osaka Univ.*, 1970, **19**, 7.
[50] T. A. Jackson, *Experientia*, 1971, **27**, 242.
[51] B. P. Thacker, *Indian J. Chem.*, 1971, **5**, 42.
[52] V. Gut and K. Poduška, *Coll. Czech. Chem. Comm.*, 1971, **36**, 3470.
[53] P. Maldonado, C. Richaud, J. P. Aune, and J. Metzger, *Bull. Soc. chim. France*, 1971, 2933.
[54] K. Ogura and G. Tsuchihashi, *Tetrahedron Letters*, 1971, 3151.
[55] H. Nakamoto, M. Aburatuni, and M. Inagaki, *J. Medicin. Chem.*, 1971, **14**, 1021.
[56] B. W. Bycroft, D. Cameron, and A. W. Johnson, *J. Chem. Soc.* (*C*), 1971, 3040.
[57] C. C. J. Culvenor, M. C. Foster, and M. P. Hegarty, *Austral. J. Chem.*, 1971, **24**, 371.
[58] K. Bevan, J. S. Davies, C. H. Hassall, R. B. Morton, and D. A. S. Phillips, *J. Chem. Soc.* (*C*), 1971, 514.

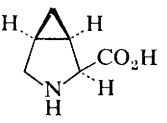
The structure of the Y base from phenylalanine t-RNA, as well as the stereochemistry at the single asymmetric centre, has been conclusively established [59] by the synthesis outlined in Scheme 4. The novel cyclopropyl




Reagents: i, $H_2$–Pd/C; ii, $Br_2$–$CHCl_3$; iii, $H_2O$

**Scheme 4**

amino-acid (14) from horse chestnuts has been synthesized by carbene addition to 3,4-dehydroproline.[60] The reaction gives rise to a mixture of the *cis*- and *trans*-isomers (14) and (15) in the ratio 1 : 3.5. The stereochemistry of the natural amino-acid (14) was established by *X*-ray crystallographic analysis.

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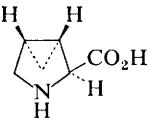
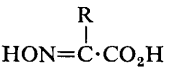



(15)

**D. *C*-Alkyl- and Substituted *C*-Alkyl-α-amino-acids.**—An improved method is claimed for the preparation of unsaturated α-amino-acids by reduction with aluminium amalgam of the unsaturated α-hydroxyimino intermediate (16) derived by the normal malonate route.[61] A convenient method for




(16) R = alkenyl

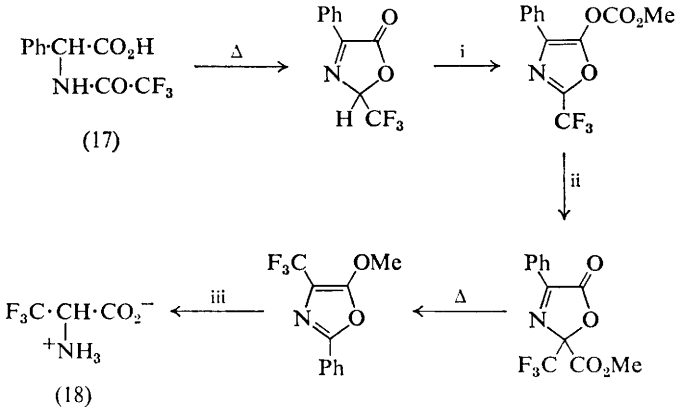
direct conversion of *N*-acyl-α-amino-acids into the *N*-acyl-αβ-dehydro-amino-acids has been reported.[62] (*S*)-α-Methyl-α-amino-acids have been obtained in high yield by a modified Strecker synthesis [63] and new syntheses of α-aminosuberic and α-aminosebacic acids have been described.[64]

Considerable interest exists in cyanoamino-acids because of the strong neurotoxic properties of the naturally occurring β-cyano-L-alanine.

[59] M. Funamizu, A. Terahara, A. M. Feinberg, and K. Nakanishi, *J. Amer. Chem. Soc.*, 1971, **93**, 6708.
[60] Y. Fujimoto, F. Irreverre, J. M. Karle, I. L. Karle, and B. Witkop, *J. Amer. Chem. Soc.*, 1971, **93**, 3471.
[61] D. J. Drinkwater and P. W. G. Smith, *J. Chem. Soc.* (*C*), 1971, 1305.
[62] J. M. Riordan and C. H. Stammer, *Tetrahedron Letters*, 1971, 4969.
[63] K. Weinges, G. Graab, D. Nagel, and B. Stemmle, *Chem. Ber.*, 1971, **104**, 3594.
[64] G. M. Shakhnazaryan, L. A. Voskanyan, and M. T. Dangyan, *Armyan. khim. Zhur.*, 1970, **23**, 709.

Dehydration of *N-o*-nitrophenylsulphenyl (Nps) asparagine and glutamine with dicyclohexylcarbodi-imide affords the corresponding protected ω-cyanoamino-acid.[65] The free amino-acids were obtained by removing the Nps group under mild conditions which did not effect the cyano-group. α-Cyanoglycine has been prepared by enzymic deacetylation of the readily available acetamidocyanoacetic acid.[65] Attempts to prepare it by chemical hydrolysis were unsuccessful because it undergoes rapid decarboxylation in hot aqueous solution.

*N*-Trifluoroacetylphenylglycine (17) has been converted by an interesting and unusual series of thermal rearrangements into trifluoroalanine (18).[66] The proposed reaction sequence is outlined in Scheme 5. The overall yield is acceptable and it is probable that this novel synthesis could be extended to other fluorinated amino-acids.



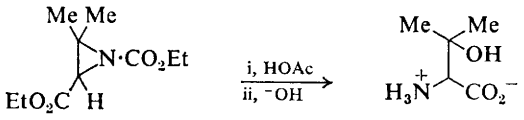

Reagents: i, $ClCO_2Me$–$NEt_3$; ii, 4-dimethylaminopyridine: iii, HBr–HOAc

**Scheme 5**

**E. α-Amino-acids with Aliphatic Hydroxy-groups in the Side-chain.**—The considerable interest in β-hydroxyvaline in relation to penicillin chemistry is reflected in the large number of syntheses already available for this compound. A further synthesis involves the addition of ethoxycarbonylnitrene to ethyl ββ-dimethylacrylate to give the aziridine intermediate (19). Ring opening of (19) with acetic acid and subsequent base hydrolysis affords (20) in good yield.[67]

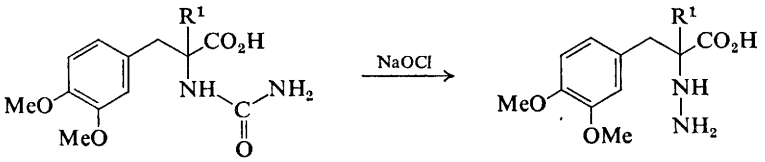
Both *erythro-* and *threo*-β-hydroxyleucine have been synthesized from β-isopropylglycidic acid,[68] and a detailed account of a number of unsuccessful routes to α-hydroxyamino-acids has appeared.[69]

[65] A. Chimiak and J. J. Pastuszak, *Chem. Ind. Internat.*, 1971, 427.
[66] G. Hofle and W. Steglich, *Chem. Ber.*, 1971, **104**, 1408.
[67] C. Berse and P. Bessette, *Canad. J. Chem.*, 1971, **49**, 2610.
[68] S. Futagawa, M. Nakahara, T. Inui, H. Katsura, and T. Kaneko, *Nippon Kagaku Zasshi*, 1971, **92**, 374.
[69] Y. Liwschitz, A. Singerman, Y. Wiesel, M. Michman, P. Braun, S. Kassel, and D. Perera, *Israel J. Chem.*, 1971, **9**, 89.

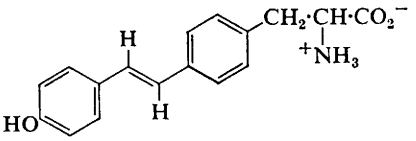



(19) (20)

**F. Aromatic and Heterocyclic α-Amino-acids.**—Aromatic amino-acids continue to be synthesized as potential enzyme inhibitors. A series of 6-alkyl-Dopa derivatives [70] and 3-(2,5-dimethoxy-4-methyl)phenylalanine [71] have been prepared by standard routes, and an improved synthesis of 6-hydroxy-Dopa is claimed.[72] A variety of tyrosine derivatives [73, 74] and 2-aminoindan-2-carboxylic acids [75] have been prepared as possible inhibitors of tyrosine hydroxylase. A novel method for the synthesis of α-hydrazino-acids related to L-Dopa [76] has been reported. The key intermediate was the hydantoic acid (21) (prepared from the amino-acid and potassium cyanate) which on treatment with sodium hypochlorite afforded the hydrazino-acid (22) in good yield. The stereochemistry at the α-centre was retained, but in all the cases so far investigated the α-centre was fully substituted. It is possible that this method may have a broader application to the synthesis of α-hydrazino-acids.




(21) (22)



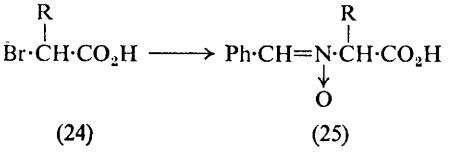

(23)

[70] A. P. Morgenstern, C. Schuijt, and W. Th. Nauta, *J. Chem. Soc.* (*C*), 1971, 3706.
[71] K. Brewster and R. M. Pinder, *J. Medicin. Chem.*, 1971, **14**, 650.
[72] F. Lee, G. H. Dickson, E. Donald, and A. A. Manian, *J. Medicin. Chem.*, 1971, **14**, 266.
[73] Y. H. Caplan, N. Zenker, D. A. Blake, and E. M. Johnson, *J. Medicin. Chem.*, 1971, **14**, 405.
[74] R. E. Counsell, P. Desai, A. Ide, P. G. Kulkarni, P. A. Weinhold, and V. B. Rethy, *J. Medicin. Chem.*, 1971, **14**, 789.
[75] R. M. Pinder, B. H. Butcher, D. A. Buxton, and D. J. Howells, *J. Medicin. Chem.*, 1971, **14**, 892.
[76] S. Karady, M. G. Ly, S. H. Pines, and M. Sletzinger, *J. Org. Chem.*, 1971, **36**, 1946, 1949.

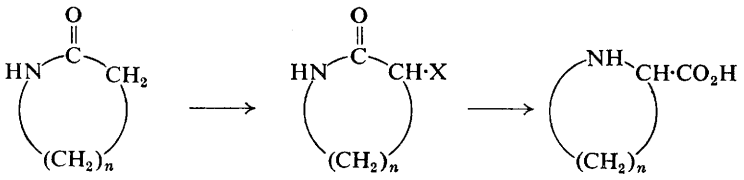
A number of fluoro-[77, 78] and amino-phenylalanines,[79] as well as *p*-azidophenylalanine,[80] designed as a photoaffinity probe in synthetic peptides, has been reported. Interest continues in structural analogues of thyroxine; the stilbene derivative (23) has been synthesized by means of a Wittig reaction.[81]

A wide range of pyridyl,[82, 83] pyridimyl,[84–86] and purinyl [87, 88] amino-acids have been synthesized mainly by well-established routes for a variety of specific reasons too diverse to enumerate. The first example of a ring-fluorinated histidine derivative has been obtained [89] by a new route involving the photochemical decomposition of diazonium fluoroborates, and the method promises to offer a general route to aromatic and heterocyclic fluorination.

**G. N-Substituted α-Amino-acids.**—A further method for the preparation of *N*-methylamino-acids, which is also claimed to give high yields and optical purity, has been reported.[90] The reaction of the L-bromo-acid (24) with *anti*-benzaldoxime afforded the *N*-oxide (25) which, on hydrogenation and hydrolysis, gave D-phenylalanine,[91] thus demonstrating that the reaction



(24) (25)



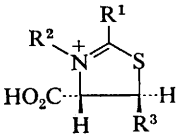
(26) (27) (28)

[77] A. T. Prudchenko, *Izvest. sibirsk. Otdel. Akad. Nauk, Ser. khim. Nauk*, 1970, 95.
[78] J. L. Fauchere and R. Schwyzer, *Helv. Chim. Acta*, 1971, **54**, 2078.
[79] I. Straukas, N. Dirvianskyte, and J. Degutis, *Zhur. org. Khim.*, 1971, **7**, 1390.
[80] R. Schwyzer and M. Caviezel, *Helv. Chim. Acta*, 1971, **54**, 1395.
[81] G. Jones and S. Wright, *J. Chem. Soc.* (*C*), 1971, 141.
[82] S. J. Scotty, P. T. Sullivan, and C. B. Sullivan, *J. Medicin. Chem.*, 1971, **14**, 211.
[83] P. T. Sullivan and S. J. Norton, *J. Medicin. Chem.*, 1971, **14**, 557.
[84] M. Y. Lidak, R. A. Paegle, M. G. Plata, and Y. P. Shvachkin, *Khim. geterotsikl. Soedinenii*, 1971, 530.
[85] R. A. Paegle, M. G. Plata, M. Y. Lidak, and Y. Y. Popel, *Khim. geterotsikl. Soedinenii*, 1971, 258.
[86] I. Y. Ulane and M. Y. Lidak, *Khim. geterotsikl. Soedinenii*, 1971, 527.
[87] D. S. Letham and H. Young, *Phytochemistry*, 1971, **10**, 23.
[88] M. Y. Lidak, Y. Y. Shluke, S. E. Poritere, and Y. P. Shvachkin, *Khim. geterotsikl. Soedinenii*, 1971, 427.
[89] K. L. Kirk and L. A. Cohen, *J. Amer. Chem. Soc.*, 1971, **93**, 3060.
[90] J. R. Coggins and N. L. Benoiton, *Canad. J. Chem.*, 1971, **49**, 1968.
[91] B. Liberek and Z. Palacz, *Roczniki Chem.*, 1971, **45**, 1173.

had proceeded with inversion of configuration at the α-centre. A novel route to α-hydrazino-acids from the corresponding amino-acid has been described in the foregoing section.[76]

An interesting variation of the Favorski reaction has been employed to synthesize a series of ring homologues of proline.[92] α-Halogenation of the readily available ω-aminolactams (26) afforded the α-halogeno-compounds (27) which underwent ring contraction on treatment with base to give the α-imino-acids (28). The already extensive programme on the synthesis of *N*-bis-2-halogenoethyl derivatives of amino-acids as potential antimetabolites has been extended.[93–95]

**H. α-Amino-acids containing Sulphur.**—Thioamides react smoothly with α-bromo-αβ-unsaturated acids to form the thiazolinium derivatives (29) which on hydrolysis yield N-substituted cysteines. The rate of thiazolinium



(29)

formation decreases with increasing number of substituents owing to unfavourable steric interactions; nevertheless the reaction offers an interesting and versatile route to substituted cysteine derivatives.[96] The syntheses of a series of *S*-alkyl-2-methyl cysteine [97] and *S*-alkylhomocystine derivatives [98] employing standard procedures have been reported.

**I. α-Amino-acids which have been Synthesized for the First Time**

| *Compound* | *Ref.* |
|---|---|
| L-3-Carboxy-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline | 15 |
| 'γ-L-Glutaminyl-4-hydroxybenzene' (*N*-γ-L-glutamyl-*p*-aminophenol) | 27 |
| *S*-(3-Hydroxy-3-carboxyethylthio)homocystine | 32 |
| *S*-(2-Hydroxy-2-carboxyethylthio)homocysteine | 32 |
| *cis*- and *trans*-3,4-methylene-L-proline | 60 |
| 3-(2-Methyl-4,5-dihydroxyphenyl)-DL-alanine | 70 |
| 3-(2-Ethyl-4,5-dihydroxyphenyl)-DL-alanine | 70 |
| 3-(2-Isopropyl-4,5-dihydroxyphenyl)-DL-alanine | 70 |
| 3-(2-t-Butyl-4,5-dihydroxyphenyl)-DL-alanine | 70 |
| 3-(2,5-Dimethoxy-4-methylphenyl)-DL-alanine | 71 |
| 3-Ethyl-α-methyl-DL-tyrosine | 74 |

[92] H. T. Nagasawa, J. A. Elberling, P. S. Fraser, and N. S. Mizumo, *J. Medicin. Chem.*, 1971, **14**, 501.
[93] G. G. Blinova and E. G. Sochilin, *Zhur. obshchei Khim.*, 1970, **40**, 2748.
[94] L. V. Alekseeva, N. L. Burde, and Z. V. Pushkareva, *Zhur. org. Khim.*, 1971, **7**, 647.
[95] G. A. Davydova, B. M. Karmanskaya, I. A. Redkin, and A. I. Tochilkin, *Khim. Farm. Zhur.*, 1971, **5**, 11.
[96] A. Eidem, K. Undheim, and K. R. Reistad, *Acta Chem. Scand.*, 1971, **25**, 1.
[97] S. Tahara and Y. Obata, *Agric. and Biol. Chem. (Japan)*, 1971, **35**, 53.
[98] A. Rinaldi and C. De Marco, *Ital. J. Biochem.*, 1971, **20**, 1.

| *Compound* | *Ref.* |
|---|---|
| 3-Isopropyl-α-methyl-DL-tyrosine | 74 |
| 3-t-Butyl-α-methyl-DL-tyrosine | 74 |
| DL-2-Amino-5-hydroxy-indan-2-carboxylic acid | 75 |
| DL-2-Amino-5-methoxy-indan-2-carboxylic acid | 75 |
| DL-2-Amino-5-carboxy-indan-2-carboxylic acid | 75 |
| DL-2-Amino-5-chloro-indan-2-carboxylic acid | 75 |
| DL-2-Amino-5-bromo-indan-2-carboxylic acid | 75 |
| DL-2-Amino-5-iodo-indan-2-carboxylic acid | 75 |
| L-α-(3,4-Dihydroxybenzyl)-α-hydrazinopropionic acid | 76 |
| L-α-(3,4-Dimethoxybenzyl)-α-hydrazinopropionic acid | 76 |
| L-α-(3,4-Dihydroxybenzyl)-α-hydrazinobutyric acid | 76 |
| L-α-(3,4-Dimethoxybenzyl)-α-hydrazinobutyric acid | 76 |
| 3-(2,4-Difluorophenyl)-DL-alanine | 77 |
| 3-(3,4-Difluorophenyl)-DL-alanine | 77 |
| 3-(3,5-Difluorophenyl)-DL-alanine | 77 |
| 3-(2,5-Difluorophenyl)-DL-alanine | 77 |
| 3-(2,6-Difluorophenyl)-DL-alanine | 77 |
| 3-(2,3,5,6-Tetrafluorophenyl)-DL-alanine | 77 |
| 3-(3,5-Dichloro-2,4,6-trifluorophenyl)-DL-alanine | 77 |
| 3-(2,3,4,5,6-Pentafluorophenyl)-DL-alanine | 78 |
| 3-(*m*-Bis-2-chloroethylaminophenyl)-DL-serine (*threo* and *erythro*) | 79 |
| 3-(*p*-Bis-2-chloroethylaminophenyl)-DL-serine (*threo* and *erythro*) | 79 |
| *p*-Azido-L-phenylalanine | 80 |
| *trans*-β-4-*p*-hydroxystyryl-DL-phenylalanine | 81 |
| β-(1,2-Dihydro-2-oxo-3-pyridyl)-DL-alanine | 82 |
| β-(1,2-Dihydro-2-oxo-4-pyridyl)-DL-alanine | 82 |
| β-(1,2-Dihydro-2-oxo-5-pyridyl)-DL-alanine | 82 |
| β-(1,2-Dihydro-2-oxo-6-pyridyl)-DL-alanine | 82 |
| β-(2-Fluoro-3-pyridyl)-DL-alanine | 82 |
| β-(2-Fluoro-5-pyridyl)-DL-alanine | 82 |
| β-(2-Fluoro-6-pyridyl)-DL-alanine | 82 |
| β-(2-Bromo-3-pyridyl)-DL-alanine | 83 |
| β-(2-Bromo-4-pyridyl)-DL-alanine | 83 |
| β-(2-Bromo-5-pyridyl)-DL-alanine | 83 |
| β-(2-Bromo-6-pyridyl)-DL-alanine | 83 |
| β-(2-Chloro-3-pyridyl)-DL-alanine | 83 |
| β-(2-Chloro-4-pyridyl)-DL-alanine | 83 |
| β-(2-Chloro-5-pyridyl)-DL-alanine | 83 |
| β-(2-Chloro-6-pyridyl)-DL-alanine | 83 |
| β-(Thymin-1-yl)-DL-alanine | 84 |
| β-(5-Fluorouracil-1-yl)-DL-alanine | 84 |
| β-(5-Bromouracil-1-yl)-DL-alanine | 84 |
| β-(5-Chlorouracil-1-yl)-DL-alanine | 84 |
| β-(Cytosin-1-yl)-DL-alanine | 84 |
| *N*-(2-Chloro-5-fluoro-4-pyrimidyl)glycine | 85 |
| *N*-(2-Chloro-5-fluoro-4-pyrimidyl)-DL-phenylalanine | 85 |
| *N*-(2-Chloro-5-fluoro-4-pyrimidyl)-DL-leucine | 85 |
| *N*-(2-Chloro-5-fluoro-4-pyrimidyl)-DL-valine | 85 |
| *N*-(2-Chloro-5-fluoro-4-pyrimidyl)-DL-tryptophan | 85 |
| *N*-(2-Ethylthio-5-fluoro-4-pyrimidyl)glycine | 85 |
| *N*-(2-Ethylthio-5-fluoro-4-pyrimidyl)-DL-phenylalanine | 85 |
| *N*-(2-Ethylthio-5-fluoro-4-pyrimidyl)-DL-leucine | 85 |
| *N*-(2-Ethylthio-5-fluoro-4-pyrimidyl)-DL-valine | 85 |
| *N*-(2-Ethylthio-5-fluoro-4-pyrimidyl)-DL-tryptophan | 85 |

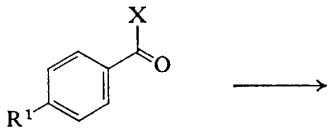
| *Compound* | *Ref.* |
|---|---|
| *N*-(2-Chloro-5-bromo-4-pyrimidyl)-DL-alanine | 86 |
| *N*-(2-Chloro-5-bromo-4-pyrimidyl)-DL- and L-leucine | 86 |
| *N*-(2-Chloro-5-bromo-4-pyrimidyl)-DL- and L-valine | 86 |
| *N*-(2-Chloro-5-bromo-4-pyrimidyl)-DL-tryptophan | 86 |
| *N*-(2-Chloro-5-bromo-4-pyrimidyl)-L-isoleucine | 86 |
| *N*-(2-Chloro-5-iodo-4-pyrimidyl)-DL-leucine | 86 |
| *N*-(2-Chloro-5-iodo-4-pyrimidyl)-DL-valine | 86 |
| *N*-(2-Chloro-5-iodo-4-pyrimidyl)-DL-alanine | 86 |
| *N*-(Purin-6-yl)-DL-α-phenylglycine | 87 |
| *N*-(Purin-6-yl)-L-leucine | 87 |
| *N*-(Purin-6-yl)-L-valine | 87 |
| DL-2-(Purin-6-yl-amino)-5-methylhex-4-enoic acid | 87 |
| $N^{\varepsilon}$-(5-Amino-6-chloro-4-pyrimidyl)lysine | 88 |
| α-Amino-ε-(6-chloro-9-purinyl)caproic acid | 88 |
| 4-Fluoro-DL-histidine | 89 |
| DL-Hexahydro-1*H*-azepine-2-carboxylic acid | 92 |
| DL-Octahydro-2-azocine carboxylic acid | 92 |
| DL-Octahydro-2-azonine-2-carboxylic acid | 92 |
| DL-Decahydro-2-azecine carboxylic acid | 92 |
| DL-Azacycloundecane-2-carboxylic acid | 92 |
| *S*-Methyl-2-methyl-DL-cysteine | 97 |
| *S*-Ethyl-2-methyl-DL-cysteine | 97 |
| *S*-Propyl-2-methyl-DL-cysteine | 97 |
| *S*-Isopropyl-2-methyl-DL-cysteine | 97 |
| *S*-Butyl-2-methyl-DL-cysteine | 97 |
| *S*-Isobutyl-2-methyl-DL-cysteine | 97 |
| *S*-t-Butyl-2-methyl-DL-cysteine | 97 |
| *S*-Amyl-2-methyl-DL-cysteine | 97 |
| *S*-Isoamyl-2-methyl-DL-cysteine | 97 |
| *S*-Allyl-2-methyl-DL-cysteine | 97 |
| *S*-(β-Aminoethyl)homocysteine | 98 |

**J. Labelled Amino-acids.**—The commercial availability of a wide range of $^{14}C$-labelled amino-acids is reflected in the noticeable drop in the publications relating to their syntheses, and those described have been performed by standard procedures such as the Strecker synthesis.[99] The chemical emphasis appears to have shifted to stereospecific syntheses of tritiated and deuteriated amino-acids and this has led to detailed examinations of the mechanisms of some of the general amino-acid syntheses.

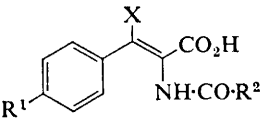
In last year's Report it was pointed out that the synthesis of labelled aldehydes of the type (30) had considerable potential for specific labelling of amino-acids at the prochiral β-centre. This potential has now been realized by two groups working independently.[100, 101] Condensation of (30) with an *N*-acylglycine derivative gave an oxazolone which opened with alkali to yield the acylaminocinnamic acid (31) of established *trans*-configuration. Catalytic hydrogenation of (31) was expected, and observed,

[99] L. Pichat, P. N. Liem, and J. P. Guermont, *Bull. Soc. chim. France*, 1971, 837.
[100] K. R. Hanson, R. H. Wightman, J. Staunton, and A. R. Battersby, *Chem. Comm.*, 1971, 185.
[101] G. W. Kirby and J. Michael, *Chem. Comm.*, 1971, 415.

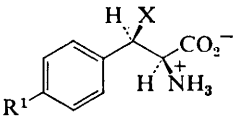
to proceed in a *syn*-stereospecific fashion. Resolution of the resulting acylamino-acid by enzymic deacylation afforded the (3*R*)-L- and (3*S*)-D-amino-acids (32) and (33) respectively. So far this method has only been applied to derivatives of phenylalanine and tyrosine but it undoubtedly has wider potential.



(30) X = $^2$H or $^3$H

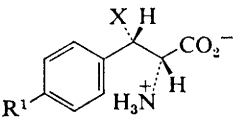


(31)



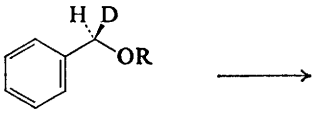
(32)

X = $^2$H or $^3$H

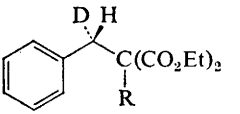


(33)

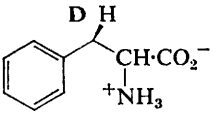
An alternative route[101,102] to some of the deuteriated derivatives described above, which has also confirmed the stereochemical assignments, employs (1*S*)-[1-$^2$H]benzyl alcohol (34). Enzymic reduction of benzaldehyde under established conditions afforded (34) and reaction of the corresponding tosylate (35) with diethyl malonate or with diethyl *N*-acetamidomalonate gave (36) and (37) respectively, which were converted by standard methods into (3*R*)-DL-[3-$^2$H]phenylalanine (38). The reaction proceeds with inver-



(34) R = H
(35) R = tosyl



(36) R = H
(37) R = NH·COMe



(38)

sion of configuration at the β-centre and the high degree of stereoselectivity has been established by physical methods and by comparison with the corresponding products from the azlactone route described above.

[102] R. Ife and E. Haslam, *J. Chem. Soc.* (*C*), 1971, 2818.

A convenient and specific general method for the preparation of α-deuteriated and α-tritiated amino-acids involves incorporation of solvent isotopic hydrogen concomitant with decarboxylation of synthetic substituted aminomalonate precursors.[103] Reaction of substituted pyruvic acids with $^{15}$N-labelled ammonia and sodium cyanohydridoborate affords a facile route to $^{15}$N-labelled amino-acids. The use of the deuteriated or tritiated cyanohydridoborate and $^{15}$N-labelled ammonia allows the synthesis of doubly labelled amino-acids.[104]

**K. Resolution of α-Amino-acids.**—A general review which analyses the problems associated with enzymic, chemical, and chromatographic methods for resolving optical isomers has appeared.[105] The separation of racemates on asymmetric sorbents continues to attract attention. A co-polymer of a chloromethylated styrene-*p*-divinylbenzene and L-proline is claimed to resolve quantitatively DL-amino-acids when used as sorbent in the presence of transition-metal ions.[106, 107] The basis of the technique is stated to be the preferential transition-metal complex formation between the L-amino-acid bound in the resin and the D-isomer in solution.

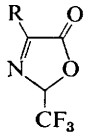
It has been known for some time that optically active stationary phases, usually dipeptides, are effective in resolving amino-acid derivatives by gas–liquid chromatography, but so far this method has been limited to analytical applications rather than preparative use; further investigations in this area have been reported.[108]

The use of carrier-bound enzymes, *i.e.* water-insoluble enzyme systems, for the resolution of amino-acids is now well established. A continuous process using carrier-bound hog acylase has been developed which is claimed to be more efficient than the normal process, since the inhibition of the enzyme is reduced by the continual removal of the reaction products.[109] The papain-catalysed reaction between racemic *N*-acylamino-acids and phenylhydrazines has been shown to proceed preferentially with the L-isomer to give the corresponding optically active hydrazide.[110] Practically 100% optical efficiency was achieved with *o*-fluorophenylhydrazine, and the method represents a novel application of the reverse of the normal proteolytic action of papain. Chymotrypsin-catalysed reactions have been employed to resolve a series of ring-substituted phenylalanine esters.[111]

Chemical methods of resolution of synthetic racemates are still commonly

[103] J. W. Thanassi, *J. Org. Chem.*, 1971, **36**, 3019.
[104] R. F. Borch, M. D. Bernstein, and H. D. Durst, *J. Amer. Chem. Soc.*, 1971, **93**, 2897.
[105] S. V. Rogozhin, *Vestnik Akad. Nauk S.S.S.R.*, 1971, **40**, 56.
[106] S. V. Rogozhin, V. A. Davankov, V. V. Korshak, V. Vesa, and A. L. Belchich, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1971, 502.
[107] S. V. Rogozhin and V. A. Davankov, *Chem. Comm.*, 1971, 490.
[108] W. Parr, J. Pleterski, C. Yang, and E. Bayer, *J. Chromatog. Sci.*, 1971, **9**, 141.
[109] T. Barth and H. Mašková, *Coll. Czech. Chem. Comm.*, 1971, **36**, 2398.
[110] J. L. Abernethy, E. Albano, and J. Comyns, *J. Org. Chem.*, 1971, **36**, 1580.
[111] J. H. Tong, C. Petitclerc, A. Diorio, and N. L. Benoiton, *Canad. J. Biochem.*, 1971, **49**, 877.

employed for routine work and details on the use of ephedrine [112] and menthol [113] have been described. An obvious limitation to the normal methods of resolution is that, if a specific enantiometer is required, then the maximum yield is only 50%. An interesting preliminary report on a possible method of converting racemates completely into either the D- or the L-enantiomer has been outlined.[114] The reaction of a racemic amino-acid with trifluoroacetic anhydride leads to the oxazolinone (39) which with dimethyl L-glutamate affords the LL-dipeptide derivative (40). Hydrolysis of the dipeptide, in the case of t-leucine, the only example so far reported, gave *ca.* 80% chemical and optical yields of the L-isomer. Whether or not this method has a wider application must await further evaluation.



(39)

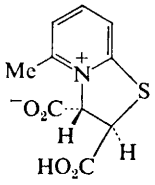
$$CF_3CO{\cdot}NH{\cdot}\underset{(L)}{CH(R)}{\cdot}CO{\cdot}NH{\cdot}\underset{(L)}{CH(CO_2Me)}{\cdot}(CH_2)_2{\cdot}CO_2Me$$

(40)

### 3 Physical and Stereochemical Studies of α-Amino-acids (See also Chapter 2, Part II, Section 2, and Part III)

**A. Crystal Structures of Amino-acids.**—The crystal structures of L-isoleucine,[115] L-tyrosine,[116] L-arginine,[117] L-cysteine,[117] DL-lysine,[117] DL-phenylalanine,[117] L-α*γ*-diaminobutyric acid [118] and the hydrochloride,[119] L-Dopa hydrochloride,[120] and DL-tryptophan formate,[121] have been described, and also those of the amides, *N*-acetyl-L-prolyl methylamide,[122] *N*-acetyl-L-methionyldimethylamide,[123] and 3,5-di-iodo-L-thyroninyl-methylamide.[124] The structure, configuration, and conformation in the crystal state of the novel amino-acid (14) have been established by an *X*-ray crystallographic analysis.[60] An analysis of the addition compound derived from bromomalic anhydride and 6-methylpyrid-2-thione has confirmed the structure (41) proposed on the basis of chemical and physical data.[125]

[112] H. Kinoshita, M. Shintani, T. Saito, and H. Kotake, *Bull. Chem. Soc. Japan*, 1971, **44**, 286.
[113] V. M. Belikov, T. F. Saveleva, and E. N. Safonova, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1971, 1461.
[114] W. Steglich, E. Frauendorfer, and F. Weygand, *Chem. Ber.*, 1971, **104**, 687.
[115] K. Torii and Y. Iitaka, *Acta Cryst.*, 1971, **B27**, 2237.
[116] A. Mostad, H. M. Nissen, and C. Rømming, *Tetrahedron Letters*, 1971, 2131.
[117] B. Khawas, *Acta Cryst.*, 1971, **B27**, 1517.
[118] P. S. Naganathan and K. Venkatesan, *Acta Cryst.*, 1971, **B27**, 2159.
[119] H. Hinazumi and T. Mitsui, *Acta Cryst.*, 1971, **B27**, 2152.
[120] R. J. Jandacek and K. M. Earle, *Acta Cryst.*, 1971, **B27**, 841.
[121] E. Bye, A. Mostad, and C. Rømming, *Acta Chem. Scand.*, 1971, **25**, 364.
[122] T. Matsuzaki and Y. Iitaka, *Acta Cryst.*, 1971, **B27**, 507.
[123] A. Aubry, M. Marraud, J. Protas, and J. Neel, *Compt. rend.*, 1971, **273**, *C*, 959.
[124] V. Cody, W. L. Duax, and D. A. Norton, *Chem. Comm.*, 1971, 683.
[125] P. Groth, *Acta Chem. Scand.*, 1971, **25**, 118.



(41)

**B. Nuclear Magnetic Resonance Spectra.**—N.m.r. spectroscopy is now an indispensable tool for structural elucidation and has played a decisive role in the characterization of many of the new amino-acids described in Section 1 (see, for example, refs. 22, 25, 28, 29, and 34). A detailed n.m.r. study on the conformation of valine and phenylalanine derivatives in different solvents, using the variation of the vicinal coupling constants to calculate changes in the population of side-chain rotamers, suggests that intramolecular interactions are more important than the dielectric constant of the medium in determining the relative energies of the rotamers.[126]

Carbamate formation in solutions of α-amino-acids and carbonate-bicarbonate, as well as the conformation of the products, has been studied by n.m.r. techniques.[127] Also conformational studies have been reported on *N*-nitroso-derivatives of sarcosine and proline,[128] and *N*-acylproline derivatives.[129]

The use of lanthanide complexes to produce large differential shifts in the $^{1}H$ and $^{13}C$ n.m.r. spectra of a wide range of organic compounds has greatly simplified the interpretation of the spectra of complex molecules. Unfortunately the use of these shift reagents has, until now, been limited to solutions of non-co-ordinating organic solvents, thus excluding the majority of amino-acids. It has now been reported that the hydrated perchlorates of europium or praeseodymium afford considerable differential shifts on the spectra of compounds in deuterium oxide.[130] So far this technique has had limited application but developments in this area could be of considerable interest.

Interest continues in the $^{13}C$ n.m.r. spectra of amino-acids,[131] but the most significant development in this area is the application of pulsed Fourier transform techniques to make use of the natural abundance of this isotope.[132] The spectra were determined with the amino-acids bound to cationic resins, and deuterium-decoupled spectra of deuteriated amino-acids showed $^{13}C$ linewidths which were significantly narrower than those

[126] R. A. Newmark and M. A. Miller, *J. Phys. Chem.*, 1971, **75**, 505.
[127] R. U. Lemieux and M. A. Barton, *Canad. J. Chem.*, 1971, **49**, 767.
[128] F. H. C. Stewart, *Austral. J. Chem.*, 1971, **24**, 1949.
[129] H. L. Maia, K. G. Orrell, and H. N. Rydon, *Chem. Comm.*, 1971, 1209.
[130] F. A. Hart, G. P. Moss, and M. L. Staniforth, *Tetrahedron Letters*, 1971, 3389.
[131] W. Voelter, G. Jung, E. Breitmaier, and E. Bayer, *Z. Naturforsch.*, 1971, **26**, 213.
[132] H. Sternlicht, G. L. Kenyon, E. L. Packer, and J. Sinclair, *J. Amer. Chem. Soc.*, 1971, **93**, 199.

from proton-decoupled, protonated amino-acids. The resin method has the advantage of shortening the spin–lattice relaxation time for quaternary carbons and other carbons that do not have hydrogens bonded to them, thus enabling these to be distinguished from carbons which are bonded to hydrogens.

The recent advances in pulsed Fourier transform techniques have allowed n.m.r. studies of $^{15}N$ in natural abundance to be undertaken, and the $^{15}N$ chemical shifts of some amino-acid methyl ester hydrochlorides have been reported.[133] There are as yet insufficient data to give more than broad generalizations, but it appears that a $\gamma$-alkyl substituent effect operates similar to that observed in $^{13}C$ spectra, and the method could have considerable potential. The abundant $^{14}N$ isotope is less useful since it possesses an electric quadrupole moment which results in considerable line-broadening, but studies on amino-acids in the solid state have established the $^{14}N$ nuclear coupling constants.[134] A further study in the solid state has determined the proton spin–lattice relaxation times as a function of temperature and has related the results to the activation energies for reorientation of the various $^{+}NH_3$ groups.[135] The first routine measurements on amino-acids of tritium magnetic resonance spectra have been reported.[136] The problems, including self-radiolysis, were discussed in detail but it is difficult, at present, to envisage any significant advantages of this technique to amino-acid chemistry.

**C. Optical Rotatory Dispersion and Circular Dichroism.**—Surveys of the o.r.d. and c.d. curves of protein amino-acids and the c.d. curves of less-common amino-acids[137] have revealed that all compounds with the L-configuration at the $\alpha$-centre give positive Cotton effects, provided that there is no other chromophoric system present. A general sector rule for $\alpha$-amino-acids has now been proposed[138] which relates the sign and amplitude of the Cotton effect with the conformation and the absolute configuration at the $\alpha$-centre. The rule is based on the octant rule and derives from the sector principle originally proposed for lactones. It depends on the basic assumption, for which there is considerable evidence, that in solution the $N—C^{\alpha}—COO$ atoms are co-planar. For the purpose of analysis the two C—O bonds in the carboxylate ion are considered as two equivalent ketone groups and the plane bisecting the carboxylate ion is taken as a symmetry plane (Figure 1, plane A). By assigning two additional planes $P_1$ and $P_2$ through the carboxylate carbon atom, each perpendicular to a C—O bond, the octant rule can be applied to each separately. The

[133] P. S. Pregosin, E. W. Randall, and A. I. White, *Chem. Comm.*, 1971, 1602.
[134] D. T. Edmonds and P. A. Speight, *Phys. Letters* (*A*), 1971, **34**, 325.
[135] R. G. C. McElroy, R. Y. Dong, M. M. Pintar, and W. F. Forbes, *J. Magn. Resonance*, 1971, **5**, 262.
[136] J. Bloxsidge, J. A. Elvidge, J. R. Jones, and E. A. Evans, *Org. Magn. Resonance*, 1971, **3**, 127.
[137] L. Fowden, P. M. Scopes, and R. N. Thomas, *J. Chem. Soc.* (*C*), 1971, 833.
[138] E. G. Jorgensen, *Tetrahedron Letters*, 1971, 863.

summation of the two effects leads to the cancellation of contributions in some 30° sectors and reinforcement in others, as illustrated in Figure 1. Since the carboxylate ion has a true plane of symmetry the corresponding sectors below the plane are of opposite sign.

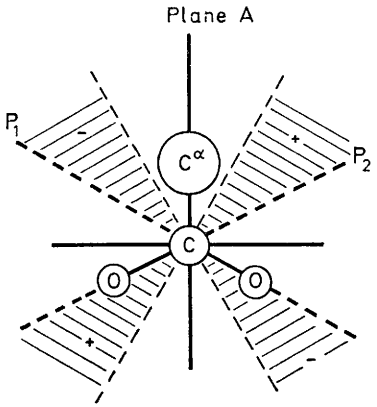



**Figure 1**

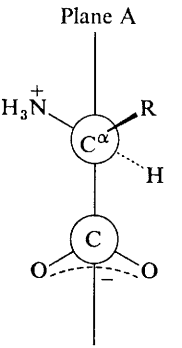



**Figure 2**

It can be seen from Figure 2 that, assuming the above-mentioned conditions apply, only the side-chain group and the α-hydrogen atom make significant contributions to the rotatory effect and that for L-α-amino-acids a greater amount of the positive sector is occupied. The rule appears to be generally applicable and rationalizes the low rotation of L-proline and the increase in rotation with increasing bulk of the side-chain, and it can be modified to account for the greater positive amplitude of L-α-amino-acids in acidic solution.

The c.d. curves for a range of α-aryl-α-amino-acids and their derivatives have been recorded. Those with the *S*-configuration all give strong positive Cotton effects and the conformations of these compounds have been discussed in relation to other α-amino-acids.[139] The o.r.d. and c.d. have also been reported for L-tyrosine,[140] L-tryptophan,[141] mono- and oligo-nucleotides[142] of L-α-amino-acids, and dithioethoxycarbonyl-L-α-amino-acids.[143]

[139] W. Klyne, P. M. Scopes, R. N. Thomas, and H. Dahn, *Helv. Chim. Acta*, 1971, **54**, 2420.

[140] E. H. Strickland, M. Wilchek, J. Horwitz, and C. Billups, *J. Biol. Chem.*, 1971, **247**, 572.

[141] H. Umeyama, T. Nagai, and H. Nogami, *Chem. and Pharm. Bull. (Japan)*, 1971, **19**, 441.

[142] E. S. Gromova, B. V. Tyaglov, and Z. A. Shabarova, *Biochim. Biophys. Acta*, 1971, **240**, 1.

[143] K. Ishikawa, K. Achiwa, and I. S. Yamada, *Chem. and Pharm. Bull. (Japan)*, 1971, **19**, 912.

**D. Mass Spectrometry.**—The continuing interest in the application of mass spectrometry to peptide sequence determination has led to further investigations on the mass spectra of amino-acid derivatives, and the spectra of methyl-,[144] phenyl-,[144] and phenylthio-hydantoins[145] as well as 2-anilino-5-thiazolinine[146] derivatives have been reported. For the same reason, chemical ionization mass spectrometry has been extended to phenythio-hydantoins. The technique leads to enhanced stability of the molecular ion and markedly reduces fragmentation, thus increasing the analytical potential of the method.[147]

Trimethylsilylation is still commonly employed to increase the volatility of amino-acids (see ref. 26) and the mass spectra of a series of trimethylsilyl derivatives of deuteriated and $^{13}C$-enriched amino-acids have been determined,[148] in relation to their possible application to biosynthetic studies. Details of the fragmentation patterns of simple amino-acids have also been recorded.[149, 150]

**E. Other Physical and Stereochemical Studies.**—A considerable amount of detail on the conformation of free amino-acids[151, 152] and their derivatives,[153–155] both in solution and the solid state, has been deduced from i.r. and Raman spectroscopy. The heats of ionization of all the commonly occurring amino-acids have been calculated from calorimetric data[156] and the heats of solution of a number determined both in light and heavy water.[157]

The determination of the relative and absolute stereochemistry is an integral part of structure elucidation of amino-acids, and physical methods have played a predominant role in establishing the chirality of a considerable number of synthetic and naturally occurring amino-acids. The separation and characterization of the diastereoisomers of threonine,[158] γ-fluoroglutamic acid,[159] and β-methyltryptophan[160] have been reported.

[144] T. Sun and R. E. Lovins, *Analyt. Biochem.*, 1971, **45**, 176.
[145] F. Weygand and R. Obermeier, *European J. Biochem.*, 1971, **20**, 72.
[146] T. Fairwell and R. E. Lovins, *Biochem. Biophys. Res. Comm.*, 1971, **43**, 1280.
[147] H. M. Fales, Y. Nagai, G. W. A. Milne, H. B. Brewer, J. T. Bronzert, and J. J. Pisano, *Analyt. Biochem.*, 1971, **43**, 288.
[148] W. J. A. Van den Heuvel, J. L. Smith, and J. S. Cohen, Proceedings of the Sixth International Symposium on Advances in Chromatography, 1970, p. 293.
[149] J. G. Lawless and M. S. Chadha, *Analyt. Biochem.*, 1971, **44**, 473.
[150] E. Stenhagen and B. A. Andersson, *Arch. Mass Spectral Data*, 1971, **2**, 146.
[151] P. K. Ponnuswamy and V. Sasisekharan, *Internat. J. Protein Res.*, 1971, **3**, 1, 9.
[152] T. Akimoto, M. Tsuboi, M. Kainosho, F. Tamura, A. Nakamura, S. Muraishi, and T. Kajuira, *Bull. Chem. Soc. Japan*, 1971, **44**, 2577.
[153] Y. Koyama, T. Shimanouchi, M. Sato, and T. Tatsumo, *Biopolymers*, 1971, **10**, 1059.
[154] J. Smolikovă, A. Vitek, and K. Bláha, *Coll. Czech. Chem. Comm.*, 1971, **36**, 2474.
[155] S. Boehm and B. Ruestow, *Studies Biophys.*, 1969, **13**, 169.
[156] M. A. Marini, R. L. Berger, D. P. Lam, and C. J. Martin, *Analyt. Biochem.*, 1971, **43**, 188.
[157] A. I. Klimov and V. I. Deshcherevskii, *Biofizika*, 1971, **16**, 556.
[158] Y. Ariyoshi and N. Sato, *Bull. Chem. Soc. Japan*, 1971, **44**, 2787.
[159] J. C. Unkeless and P. Goldman, *Mol. Pharmacol.*, 1971, **7**, 293.
[160] K. F. Turchin, M. N. Preobrazhenskaya, Yu. N. Sheinker, and N. N. Suvorov, *Zhur. org. Khim.*, 1971, **7**, 1290.

An additional chemical correlation between viomycidine and capreomycidine has provided further confirmation for the relative and absolute stereochemistry of these guanidine amino-acids.[161]

## 4 Chemical Studies of Amino-acids

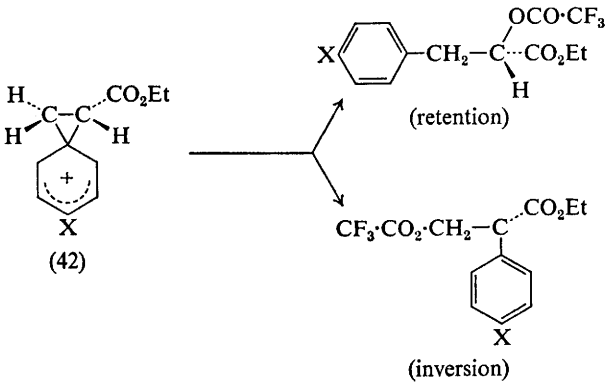
**A. Introduction.**—The first volume of what is intended as a continuous series of reviews on the general chemistry and biochemistry of amino-acids, peptides, and proteins has been published.[162] Although the first issue contained only a limited amount on amino-acids (assignment of configuration), the series offers a further source of information on amino-acid chemistry. Each year a great deal of research is published on the chemistry of amino-acids and their derivatives, much of it in relation to peptide synthesis, and this is presented elsewhere in this Report; the remainder is undertaken for a wide variety of reasons and it is therefore inevitable that this section is something of a miscellany.

**B. General Reactions.**—When amino-acids are heated at 1000 °C the predominant pyrolysis product is hydrogen cyanide;[163] in some cases the amino-nitrogen is almost quantitatively converted into hydrogen cyanide. At lower temperatures phenylalanine and tryptophan afford a mixture of polynuclear hydrocarbons and heterocyclic compounds.[164] A detailed investigation on the thermal decomposition employing differential scanning calorimetry has allowed thermolysis pathways to be proposed for a number of amino-acids in terms of the resolved thermograms.[165]

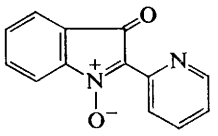
The stereochemistry and product distribution on nitrous acid deamination of L-phenylalanine, and of *p*-substituted phenylalanine ethyl esters in trifluoroacetic acid, suggests[166] that the reaction proceeds predominantly through the phenonium ion (42) as outlined in Scheme 6.

The deamination of a series of aminocycloalkane carboxylic acids has been reported;[167] the results were discussed in relation to the possible conformation of the ring systems. Strecker degradation of amino-acids with benzil afforded the expected aldehydes and tetraphenylpyrazine, which is claimed to arise by self-condensation of the intermediate 2-amino-2-phenylacetophenone.[168] Isatogen derivatives of the type (43) react with α-amino-acids in a similar manner to ninhydrin and isatin, causing oxidative deamination and decarboxylation to the aldehydes and isatogen reduction products.[169]

[161] C. Gallina, C. Marta, C. Colombo, and A. Romeo, *Tetrahedron*, 1971, **27**, 4681.
[162] 'Chemistry and Biochemistry of Amino-acids, Peptides, and Proteins', ed. B. Weinstein, Marcel Dekker, New York, 1971.
[163] W. R. Johnson and J. C. Kang, *J. Org. Chem.*, 1971, **36**, 189.
[164] J. M. Patterson, W. Y. Chen, and W. J. Smith, *Tobacco Sci.*, 1971, **15**, 41.
[165] P. G. Olafsson and A. M. Bryan, *Geochim. Cosmochim. Acta*, 1971, **35**, 327.
[166] K. Koga, C. C. Wu, and S. Yamada, *Tetrahedron Letters*, 1971, 2283.
[167] R. J. W. Cremlyn, R. M. Ellam, and T. K. Mitra, *J. Chem. Soc.* (*C*), 1971, 1647.
[168] A. F. Al-Sagyab, A. T. Atto, and F. Y. Sarah, *J. Chem. Soc.* (*C*), 1971, 3260.
[169] M. Hooper and J. W. Robertson, *Tetrahedron Letters*, 1971, 2139.




**Scheme 6**




(43)

The generally accepted mechanism for the photo-decarboxylation of 2,4-dinitrophenyl-α-amino-acids,[170] which involves decarboxylation with intramolecular oxygen transfer from the nitro-group, has been questioned.[171] It is pointed out that such a mechanism cannot explain the photo-decarboxylation in the solid state when the nitro-group is unaffected. Furthermore, it has been demonstrated that *N*-*p*-nitrophenylvaline is rapidly photo-decarboxylated to give isobutyraldehyde and *p*-nitro-aniline,[171] which suggests that the initial process is decarboxylation without oxygen transfer.

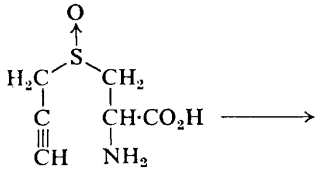
A generally efficient azeotropic method for the esterification of amino-acids has been reported.[172] The reduction of amino-acid esters proceeds more readily with lithium dimethoxyaluminium hydride than with lithium aluminium hydride itself,[173] and the esters also react with borane, trichloro-borane, and trifluoroborane to form adducts which can be converted into the corresponding N-substituted borazines.[174] A kinetic investigation extending further the programme on the Dakin–West reaction has provided evidence for the proposed pathway for the rearrangement of *N*-acyl-*s*-amino-acids.[175]

[170] O. Meth-Cohn, *Tetrahedron Letters*, 1970, 1235.
[171] P. H. MacFarlane and D. W. Russell, *Tetrahedron Letters*, 1971, 725.
[172] A. K. Saund and N. K. Mathur, *Indian J. Chem.*, 1971, **9**, 936.
[173] E. F. Rothgery and L. F. Hohnstedt, *Inorg. Chem.*, 1971, **10**, 181.
[174] M. Dymicky, E. F. Mellon, and J. Naghski, *Analyt. Biochem.*, 1971, **41**, 487.
[175] R. Knorr and G. K. Staudinger, *Chem. Ber.*, 1971, **104**, 3621, 3633.

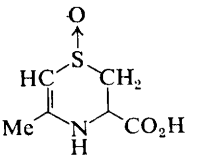
Amino-acids continue to be used as convenient chiral reagents both in synthesis and chromatography. The asymmetric synthesis of the alkaloid (+)-mesembrine has been achieved *via* an intermediate L-proline derivative.[176] Enantioselective Raney nickel catalysts have been prepared with amino-acids [39, 177] and amino-acid derivatives have been employed as stationary phases in ion-exchange [106, 107] and gas–liquid chromatography.[108]

**C. Specific Reactions.**—A systematic investigation of the desulphurization of sulphur-containing amino-acids with Raney nickel has established that cysteine and cystine can be completely desulphurized under relatively mild conditions, whereas methionine is essentially unchanged.[178] Similar results have been obtained using phosphorous acid, which has been used successfully with glutathione and oxytocin.[179]

The alkynyl cysteine-*S*-oxide (44) and the corresponding dioxide, in the presence of base, undergo an internal addition of the amino-function to the triple bond to give a cyclic sulphoxide (45) and a cyclic sulphone respectively.[180] Bisulphite has been shown to catalyse the aerial oxidation of methionine to the *S*-oxide [181] and a detailed investigation of thiazolidine formation from L-cysteine and formaldehyde has been reported.[182] *S*-Acetamido-methylcysteine can be directly oxidized to cystine derivatives with iodine,[183] and the method has been applied to the synthesis of the previously unknown cyclo-L-cystine (46).



(44)



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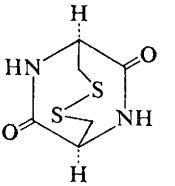
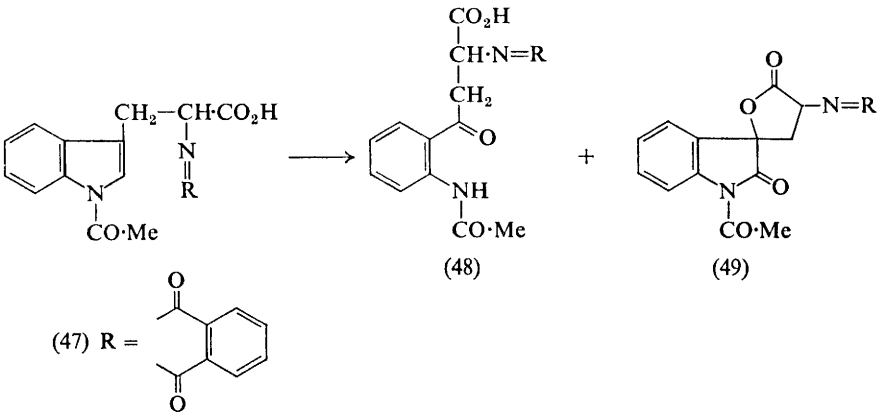


(46)

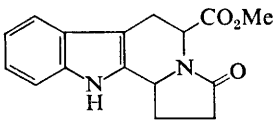
1-Acetyltryptophan has been synthesized from tryptophan *via* the *N*-phthaloyl derivative (47); surprisingly this compound had not previously been reported in the literature.[184] Oxidation of (47) with chromium trioxide afforded the kynurenine derivative (48) and the unusual dioxindole lactone (49).

[176] S. Yamada and G. Otani, *Tetrahedron Letters*, 1971, 1133.
[177] F. Higashi, T. Ninomiya, and Y. Izumi, *Bull. Chem. Soc. Japan*, 1971, **44**, 1333.
[178] M. T. Perlstein, M. Z. Atassi, and S. H. Cheng, *Biochem. Biophys. Acta*, 1971, **236**, 174.
[179] C. Ivanov and C. O. Ivanov, *Doklady Bolg. Akad. Nauk*, 1971, **23**, 1365.
[180] J. F. Carson and L. E. Boggs, *J. Org. Chem.*, 1971, **36**, 611.
[181] M. Inoue and H. Hikoya, *Chem. and Pharm. Bull. (Japan)*, 1971, **19**, 1286.
[182] R. G. Kallen, *J. Amer. Chem. Soc.*, 1971, **93**, 6227, 6236.
[183] B. Kamber, *Helv. Chim. Acta*, 1971, **54**, 927.
[184] S. Ohki and T. Nagasaka, *Chem. and Pharm. Bull. (Japan)*, 1971, **19**, 545, 603.



(47) R =

Tryptophan methyl ester reacts smoothly with 3-pyrrolin-2-one to yield (50); the stereochemical course of the reaction is not defined but it is suggested that the reaction could be employed for modifiying peptide structures.[185]



(50)

A new mild reductive cleavage of acyl-guanidines to amines has been demonstrated by the conversion of arginine into ornithine.[186] The reaction of nitromalondialdehyde with arginine in aqueous alkaline media results in the quantitative formation of δ-(5-nitro-2-pyrimidyl)ornithine.[187] The reaction may have potential in peptide sequence work and in mass spectrometry for increasing the volatility of guanidine-containing amino-acids.

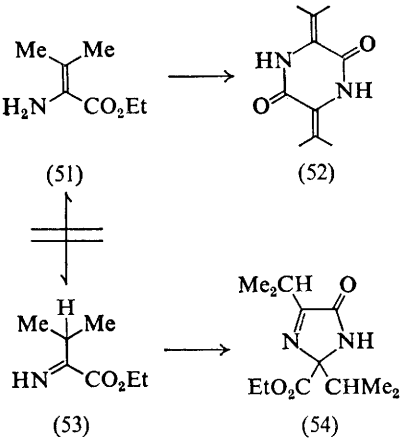
The *N*-oxides of *NN*-alkylamino-acids readily decarboxylate when heated with toluene-*p*-sulphonyl chloride in pyridine to yield the secondary amine and formaldehyde,[188] presumably by the mechanism outlined in Scheme 7.

The αβ-dehydrovaline ester (51), prepared by reduction of the corresponding α-nitro-ester, dimerizes on heating[189] to give the diketopiperazine (52), whereas the α-imino-ester (53), prepared from the α-keto-ester by the

[185] V. Bocchi, G. Casnati, and G. P. Gardini, *Tetrahedron Letters*, 1971, 683.
[186] J. S. Bland and J. F. W. Keana, *Chem. Comm.*, 1971, 1024.
[187] A. Signor, G. M. Bonora, L. Biondi, D. Nisato, A. Marzotto, and E. Scoffone, *Biochemistry*, 1971, **10**, 2748.
[188] Y. Ikutani, *Bull. Chem. Soc. Japan*, 1971, **44**, 271.
[189] C. Shin, M. Masaki, and M. Ohta, *Bull. Chem. Soc. Japan*, 1971, **44**, 1657.

addition of *N*-phenyltriphenylphosphinimine, cyclizes at room temperature to the imidazolidone (54). It is suggested that under the conditions of the two reactions there is no interconversion between (51) and (53).

**Scheme 7**

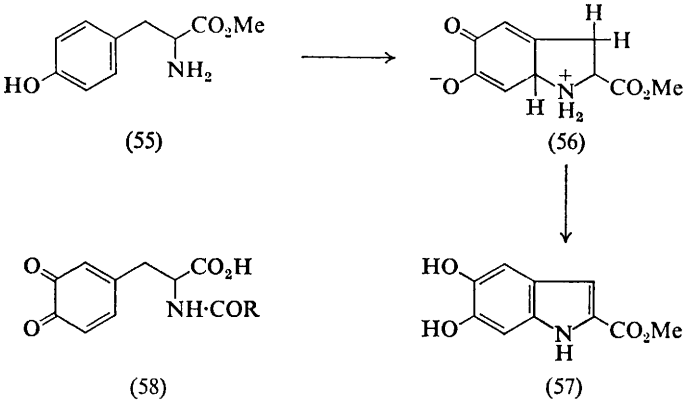


A novel synthesis of D-ribose has been reported which employs as the starting material L-glutamic acid, the asymmetric α-centre of which is subsequently converted into C-4 of D-ribose.[190]

**D. Non-enzymic Models of Biochemical Processes Involving Amino-acids.**— The chemical oxidation of tyrosine with potassium nitrosodisulphonate (Fremy's salt) has been found to follow the proposed enzymic pathways. Tyrosine methyl ester (55) is oxidized to 2-carboxy-5,6-dihydroxyindole (57) through the intermediacy of (56), which possesses the characteristic spectral properties of the enzyme-catalysed intermediate for amino-terminal tyrosine peptides. Carboxy-terminal peptides are oxidized by Fremy's salt to the *o*-quinone intermediate (58) which on reduction affords 3,4-dihydroxyphenylalanine derivatives, again paralleling the enzymic pathway.[191]

The stability of organically bound nitrogen in soil humic acids has been attributed to combination of amino-acids, peptides, and proteins with quinones. In order to investigate the chemical ability of this type of compound the synthesis of a number of benzoquinones containing α-amino-acid esters has been described.[192] Model reactions involving the

[190] K. Koga, M. Taniguchi, and S. Yamada, *Tetrahedron Letters*, 1971, 266.
[191] S. Dukler, M. Wilchek, and D. Lavie, *Tetrahedron*, 1971, **27**, 607.
[192] P. A. Cranwell and R. D. Haworth, *Tetrahedron*, 1971, **27**, 1831.



(55) (56) (58) (57)

direct cleavage of diphenyl ether linkages, in studies related to the metabolism of thyroxine, have been documented.[193] The non-enzymic reaction of L-serine with indole and hydrogen sulphide, in the presence of pyridoxal, to give DL-tryptophan and DL-cystine respectively, is reported to proceed through an intermediate pyridoxylidene amino-acrylic acid derived by $\beta$-elimination of the Schiff base.[194]

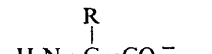
A synthetic chiral biphenyl derivative is stated to act as a stereospecific catalyst for the racemization of amino-acids.[195] Tropic acid with the *S*-configuration, *i.e.* naturally occurring tropic acid, has been obtained from L-phenylalanine by nitrous acid deamination, and the reaction is claimed to proceed by a route similar to the biochemical process.[196]

**E. Effects of Electromagnetic Radiation on Amino-acids.**—The radicals produced from amino-acids continue to be studied under various conditions. Both radiolysis and chemical techniques have been employed for the formation of radicals which were subsequently studied by e.s.r. spectroscopy, and it is important to note that in cases in which a comparison of the results from both methods is possible the e.s.r. spectra obtained are essentially identical. E.s.r. studies on radicals produced by radiolysis support the generally established pathways for the reaction of amino-acids with •OH radicals and hydrated electrons,[197–200] and in the aqueous alkaline solution of all the amino-acids investigated, radicals of the type (59) were observed.[197] Similar observations were made on the radicals produced with $Ti^{3+}$–$H_2O_2$ at pH 9—12, and in this case the temperature-dependent proton hyperfine splitting from the amino-hydrogens was noted and interpreted in terms of

[193] T. Matsuura, T. Nagamachi, and A. Nishinaga, *J. Org. Chem.*, 1971, **36**, 2016.
[194] K. Korte and U. Schmidt, *Monatsh.*, 1971, **102**, 207.
[195] K. Hirota and Y. Izumi, *Bull. Chem. Soc. Japan*, 1971, **44**, 2287.
[196] K. Koga, C. C. Wu, and S. Yamada, *Tetrahedron Letters*, 1971, 2287.
[197] R. W. Fessenden and P. Neta, *J. Phys. Chem.*, 1971, **75**, 738.
[198] H. C. Box and E. E. Budzinski, *J. Chem. phys.*, 1971, **55**, 2446.
[199] G. Lassman and W. Damerau, *Studies Biophys.*, 1969, **17**, 195.
[200] V. T. Srinivasan and A. Van de Vorst, *Internat. J. Radiation Biol.*, 1971, **19**, 133.

the non-coplanarity of the amino-group with the nodal plane of the $\pi$-system.[201]

The radicals (60) and (61), which have different e.s.r. spectra, are formed from the $\cdot OH$ radical and glycine in aqueous solution; exchange between the two radicals can be induced by addition of phosphoric acid.[202]



(59)

(60)

(61)

Radiolysis of oxygenated cysteine[203] and cysteine formate[204] solutions has provided further evidence for the radiolytic pathways involving sulphur radicals. The major volatile products formed by radiolysis of *S*-n-propyl-L-cysteine sulphoxide and *S*-allyl-L-cysteine sulphoxide have been identified by a combination of g.l.c. and mass spectrometry.[205]

Flash spectrophotometry has been employed to follow the reactions of the hydrated electron with aromatic amino-acids and the results accord well with those obtained from pulse radiolysis.[206]

The photo-luminescence of aromatic amino-acids is still being extensively investigated,[207–210] usually with the object of applying the results to the elucidation of protein structure. The photolysis of tryptophan and tryptophan derivatives has attracted considerable attention; evidence has been presented which suggests that the primary photochemical reaction is N—H bond fission.[211] Photo-oxidation of tryptophan in aqueous solution at pH 6—9 affords *N*-formyl-kynurenine,[212, 213] but in dilute ammonia the main product is 4-(2-amino-2-carboxyethyl)quinazoline, which is not formed *via* formyl-kynurenine.[213] The rates of the flavin-sensitized photo-oxidation of tryptophan and of tyrosine have been determined,[214] and the role of the triplet state in their photo-ionization has been discussed.[215]

The general photochemically induced deamination of amino-acids has been investigated in detail.[216]

[201] R. Poupko, A. Loewenstein, and B. L. Silver, *J. Amer. Chem. Soc.*, 1971, **93**, 580.
[202] H. Paul and H. Fischer, *Helv. Chim. Acta*, 1971, **54**, 485.
[203] J. P. Barton and E. J. Packer, *Internat. J. Radiation Phys. Chem.*, 1970, **2**, 159.
[204] M. Morita, K. Sasai, M. Tajima, and M. Fujimaki, *Bull. Chem. Soc. Japan*, 1971, **44**, 2257.
[205] H. Nishimura, J. Mizutani, Y. Obata, and M. Namiki, *Tetrahedron*, 1971, **27**, 307.
[206] B. Finnstrom, *Photochem. and Photobiol.*, 1971, **13**, 375.
[207] E. Leroy, H. Lami, and G. Laustriat, *Photochem. and Photobiol.*, 1971, **13**, 411.
[208] P. G. Bogach, V. L. Zima, and A. M. Filenko, *Biofizika*, 1971, **16**, 340.
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[210] J. Tournon and M. A. El-Bayoumi, *J. Amer. Chem. Soc.*, 1971, **93**, 6396.
[211] M. T. Pailthorpe and C. H. Nicholls, *Photochem. and Photobiol.*, 1971, **14**, 135.
[212] R. S. Asquith and D. E. Rivett, *Biochim. Biophys. Acta*, 1971, **252**, 111.
[213] W. E. Savige, *Austral. J. Chem.*, 1971, **24**, 1285.
[214] F. Y. H. Wu and D. B. McCormick, *Biochim. Biophys. Acta*, 1971, **236**, 479.
[215] E. E. Fesenko, *Studies Biophys.*, 1968, **9**, 155.
[216] A. Riva and R. Cultrera, *Ann. Chim. (Italy)*, 1971, **61**, 624.

## 5 Analytical Methods (See also Chapter 2, Part I, Section 2A)

The pattern established in previous Reports for this section is maintained; the majority of references are cited under the appropriate heading without discussion and only the pertinent advances are presented in more detail. The improvements and developments of technique for amino-acid analysis in relation to structural studies on proteins and peptides are also covered. A book describing recent developments in analytical methods has been published.[217]

**A. Gas–Liquid Chromatography.**—The wealth of literature relating to g.l.c. of derivatized amino-acids emphasizes the developing potential of this truly quantitative method. The great advantages of the technique are speed and sensitivity. The instrumentation is simple and the resolution is such that even optical isomers can be separated on an asymmetric support.[218–220] The problems encountered with derivatization have now been largely overcome and more convenient methods for sample handling and quantitative preparation with a minimum of manipulation are being developed.[221] It is perhaps significant that the methods employed to investigate the amino-acid content of extra-terrestrial material were predominantly g.l.c. methods (see Section 1A), and arising out of this work a g.l.c. technique for nanogramme amounts of amino-acids is now available.[222] Several papers report that the effective resolution of all twenty protein amino-acids has been documented,[223–225] establishing that the problems encountered with resolving histidine, arginine, tryptophan, and cysteine have now been largely overcome. A number of other papers relating to g.l.c. of amino-acids in general have been published [226–230] and the increasing application of the method for biological materials is evident.[231–234] Chromatography of phenyl- and methyl-hydantoin derivatives also would appear to be promising [235] and the

[217] A. Niederwieser and G. Pataki, 'New Techniques in Amino-acid, Peptide, and Protein Analysis', Ann Arbor-Humphrey Sci. Publishers, Ann Arbor, Michigan, 1971.
[218] B. Feibush, *Chem. Comm.*, 1971, 544.
[219] W. Parr and P. Howard, *Chromatographia*, 1971, **4**, 162.
[220] J. C. Dabiowiak and D. W. Cooke, *Analyt. Chem.*, 1971, **43**, 791.
[221] J. M. L. Mee and C. C. Brooks, *J. Chromatog.*, 1971, **62**, 138.
[222] R. W. Zumwalt, K. Kuo, and C. W. Gehrke, *J. Chromatog.*, 1971, **55**, 267; **57**, 193.
[223] C. W. Gehrke, K. Kuo, and R. W. Zumwalt, *J. Chromatog.*, 1971, **57**, 209.
[224] C. W. Gehrke, R. W. Zumwalt, and K. Kuo, *J. Agric. Food Chem.*, 1971, **19**, 605.
[225] C. W. Moss, M. A. Lambert, and F. J. Diaz, *J. Chromatog.*, 1971, **60**, 134.
[226] B. C. Pettit and J. E. Stouffer, ref. 148, p. 273.
[227] E. Gil-Av, W. Parr, C. Yang, and E. Bayer, ref. 148, p. 287.
[228] J. Metz, W. Ebert, and H. Weicker, *Chromatographia*, 1971, **4**, 259.
[229] D. A. Shearer and R. M. Warner, *Internat. J. Environ. Analyt. Chem.*, 1971, **1**, 11.
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[231] F. J. Diaz, C. W. Moss, and M. A. Lambert, *Rev. Asoc. bioquim. argentina*, 1971, **36**, 67.
[232] J. L. Laseter, J. D. Weete, A. Albert, and C. H. Walkinshaw, *Analyt. Letters*, 1971, **4**, 671.
[233] J. E. R. Schultz, *Column*, 1971, **13**, 5.
[234] E. D. Pellizzari, C. Rising, J. H. Brown, R. W. Farmer, and L. F. Fabre, *Analyt. Biochem.*, 1971, **44**, 312.
[235] J. J. Pisano, T. J. Bronzert, and H. B. Brewer, *Analyt. Biochem.*, 1972, **45**, 43.

radiochromatographic assay of the optical purity of $^{14}C$-labelled amino-acids has been described.[236]

**B. Ion-exchange Chromatography.**—Two review articles on ion-exchange techniques have been published [237, 238] and various aspects of quantification of automatic analyser data reported.[239–242] The effect of conditions of preparation of resins on their resolution efficiency,[243] the use of Chelex-100,[244] and the separation of acidic amino-acids have also been investigated.[245] A modified pyridine–formic acid solvent system is recommended for use with radioactive compounds; no initial desalting is necessary but the resolution is not as good as with citrate buffers.[246] The hydrolysis of proteins and peptides in the presence of tritiated hydrochloric acid affords a simple and novel means of estimating the degree of racemization.[247]

It has been suggested that in order to avoid errors in quantitative work it is necessary to determine the colour constants with hydrindatin-ninhydrin for every amino-acid of interest.[248] The use of titanous chloride with ninhydrin instead of stannous chloride is claimed to give better colorimetric analysis [249] and further details on the detection of amino-acids with ninhydrin have been reported.[250, 251] Further work on the use of 2,4,6-trinitrobenzenesulphonic acid for the quantitative determination of amino-acids has been described.[252]

**C. Thin-layer Chromatography.**—Amino-acid derivatives which do not react readily with ninhydrin can be detected on t.l.c. by exposing the chromatogram to bleaching powder and hydrochloric acid and subsequently spraying with starch–potassium iodide solution.[253] Further improved techniques for the t.l.c. of free amino-acids have been

[236] A. V. Barooshian, M. J. Lautenschleger, and W. G. Harris, *Analyt. Biochem.*, 1971, **44**, 543.
[237] Y. Watanabe and N. Hamada, *Kagaku Tokogyo*, 1971, **45**, 17.
[238] A. N. Kosharov and M. A. Marina, *Nov. Metody Modif. Biokhim. Issed. Zhivotnovod*, 1970, 36.
[239] W. F. Kwolek and J. F. Cavins, *J. Assoc. Offic. Analyt. Chemists*, 1971, **54**, 1283.
[240] L. S. Bates, *Analyt. Biochem.*, 1971, **41**, 158.
[241] E. Welte, E. Przemeck, and M. C. Nuh, *Z. Pflanzennernaehr. Bodenk.*, 1971, **128**, 243.
[242] J. P. Ellis and J. M. Prescott, *J. Chromatog.*, 1971, **61**, 152.
[243] J. Rahm, H. Weinova, and Z. Prochazka, *J. Chromatog.*, 1971, **60**, 256.
[244] J. Boisseau and P. Jouan, *J. Chromatog.*, 1971, **54**, 231.
[245] A. Smith, P. J. Peterson, and L. Fowden, *J. Chromatog.*, 1971, **62**, 144.
[246] M. Reford-Ellis and M. N. Kelson, *J. Chromatog.*, 1971, **59**, 434.
[247] J. M. Manning, *J. Amer. Chem. Soc.*, 1970, **92**, 7449.
[248] M. Yamamoto and J. L. Lowell, *J. Chromatog.*, 1971, **57**, 152.
[249] L. B. James, *J. Chromatog.*, 1971, **59**, 178.
[250] K. Samejima, W. Dairman, and S. Udenfriend, *Analyt. Biochem.*, 1971, **42**, 222.
[251] K. Samejima, W. Dairman, J. Stone, and S. Udenfriend, *Analyt. Biochem.*, 1971, **42**, 237.
[252] K. T. Kossmann, *Aerztl. Lab.*, 1971, **17**, 375.
[253] Y. Ariyoshi, N. Sato, H. Zenda, and K. Adachi, *Bull. Chem. Soc. Japan*, 1971, **44**, 2558.

described.[254, 255] The chromatography of dinitrophenylamino-acids on thin layers of proteins is claimed to give good results.[256] Conditions for the effective separation of the dinitrophenyl derivatives of arginine and lysine,[257, 258] and for the rapid separation of phenylthiohydantoins,[259] have been reported.

**D. Other Methods.**—A theoretical study of the paper electrophoretic separation of amino-acids gives results in good agreement with experimental observations.[260] Further details of instrumentation [261] and of the applications of paper electrophoresis to amino-acids [262] have been reported. Many other topics in the analytical chemistry of amino-acids have been discussed, including polarographic determinations in water [263] and in DMSO,[264] partition chromatography,[265, 266] fluorometric measurements,[267] enzyme electrode probes for D-amino-acids,[268] and the detection of sulphur-containing amino-acids.[269]

**E. Determination of Specific Amino-acids.**—Papers on the determination of the following amino-acids have appeared: glutamic and aspartic acids,[270] tryptophan,[271–273] proline,[274] hydroxyproline,[275] cystine,[276] methionine,[277, 278] lysine,[278, 279] ornithine,[280] histidine,[281] and thyroxine.[282]

[254] J. G. Heathcote, D. M. Davies, C. Haworth, and R. W. A. Oliver, *J. Chromatog.*, 1971, **55**, 377.
[255] A. F. Krivis, *Microchem. J.*, 1971, **16**, 391.
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[265] Y. Tanimura, *Farumashia*, 1971, **7**, 628.
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[267] S. R. Nahorski, *Analyt. Biochem.*, 1971, **42**, 136.
[268] G. G. Guilbault and E. Hrabanova, *Analyt. Chim. Acta*, 1971, **56**, 285.
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[273] T. Devenyi, J. Bati, and F. Fabian, *Acta Biochim. Biophys. Acad. Sci. Hung.*, 1971, **6**, 133.
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[275] D. Dabev and H. Struck, *Biochem. Med.*, 1971, **5**, 17.
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[277] G. M. Ellinger and R. H. Smith, *Biochem. J.*, 1971, **124**, 151.
[278] S. Ferenczi, J. Bati, and T. Devenyi, *Acta Biochim. Biophys. Acad. Sci. Hung.*, 1971, **6**, 123.
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[282] N. N. Nihei, M. C. Gershengorn, T. Mitsuma, L. R. Stringham, A. Cordy, B. Kuchmy, and C. S. Hollander, *Analyt. Biochem.*, 1971, **43**, 433.